International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 7, Issue 3, Jun 2017, 55-66

TRANS STELLAR • Journal Publications • Research Consultancy

IMPACT OF SPAWN SUBSTRATES ON SPOROPHORE DEVELOPMENT AND YIELD OF MILKY MUSHROOM (*CALOCYBEINDICA)*

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ABSTRACT

The yield of Milky Mushroom (Calocybeindica) depends on quality of spawn. Experiment was conducted to find out the effect of spawn produced on different grains and their effects on sporophore development and yield of milky mushroom. Among seven spawn substrates viz., wheat, jowar, ragi, maize, paddy and bajra, jowar grain based spawn was found to be the best spawn substrate as compared to other substrates studied with to spawn run period, size (lenghth of stipe, diameter of stipe, pileus, weight of sporophore, yield and biological efficiency. The significantly higher yield (1586.21 g/bed) with maximum biological efficiency (105.74%) was obtained by the jowar grain based spawn.

KEYWORDS: *Stipe, Diameter of Stipe, Pileus & Weight of Sporophore*

Received: Feb 24, 2017; **Accepted:** Mar 20, 2017; **Published:** Apr 22, 2017; **Paper Id.:** IJASRJUN20178

Original Article

INTRODUCTION

Mushrooms provide a rich addition to the diet in the form of protein, carbohydrates, valuable salts, minerals and vitamins. As food, the nutritional value of mushrooms lies between meat and vegetables. Like other vegetables it contains about 90 per cent moisture (Crisian and Sands, 1978) and is basically low calorie food (25-30 calorie / 100 gm fresh weight).

Milky mushroom (*Calocybeindica*) has become the third commercially grown in India after button and oyster mushrooms. This mushroom is also called White summer mushroom and DudhChhatta. This is a tropical mushroom which is a seven months crop from March – September. This mushroom was first collected in wild form from West Bengal (India) by Purkayastha and Chandra in 1974. Production technology of *Calocybe indica* has been introduced by Purkayastha and Nayak in 1979 which was improved by Purkayastha and Nayak in 1981. This mushroom is gaining popularity due to its attractive robust, white sporocarps long shelf life and taste (Chadha and Sharma, 1995). Mature sporophore of *Calocybeindica*contains 4% soluble sugars, 2.9% starch and 7.4% ash (Doshi*et al*., 1988).

Paddy straw mushroom *(Volvariellasp)* and oyster mushroom *(Pleurotussp)* could not perform better in the summer months because of their temperature preferences. Milky mushroom *(Calocybeindica)* with its ability to grow fairly at high temperatures seen to be the best alternative to such mushroom. *C. indica* with a high biological efficiency (>90%) has the ability to grow at a temperature of above 30° C and produce attractive white sporophore with excellent shelf life. These qualities make cultivatiom of milky mushroom most suitable in tropical and sub-tropical climate (Senthilnambi*et al*., 2011). This mushroom has an immense potential in production in the plain region of India due to its better temperature tolerance (30-35° C). Due to variation in temperature, button mushroom cannot be cultivated throughout the year and can be exploited during lean period. Due to these

promising characters the current study mainly focused on finding the suitability of different grown as spawn substrates and their effects sporophore development and yield of *C. indica*.

MATERIALS AND METHODS

Experiment was carried out in Mushroom Production Unit, Department of Plant Pathology, Birsa Agricultural University, Ranchi to know the performance of spawn substrates in relation to quality spawn production on different grains as spawn substrates and their effect on sporophore development and yield of *Calocybeindica.* An experiment was conducted during summer season, 2012 in Completely Randomized Design (CRD) under room conditions. There were six treatments each with four replications. The treatments included were as:

T_1 – Wheat grain, T_2 – Jowar grain, T_3 – Ragi grain, T_4 – Maize grain, T_5 – Paddy grain, T_6 – Bajra grain

Spawn Preparation

All grains were initially pre-weeded by soaking in fresh water for 12 hours and washed 2-3 times under running water. The grains were boiled separately for 15 minutes to the extent that no starch was released. Excess water was drained off and the boiled grains were spread over a clean working table to dry slightly. Then calcium carbonate (lime) @ 6 gm/kg and calcium sulphate (gypsum) @ 12 gm/kg of grains were mixed with grains. The gypsum and lime prevents the sticking of grains together and adjusts the pH (6.5 to 6.7). Each of the ready mixture was then filled in 27 × 20 cm size polypropylene bags @ 300 g approximately in each and plugged with non-absorbent cotton. All the filled and plugged bags were sterilized in a autoclave at 20 pound pressure for two hours. Next day in the morning sterilized bags were taken out and allowed to cool down for 20-30 minutes. These cooled bags were transferred in inoculation chamber. Inner surface of laminar air flow, inoculating needle and spirit lamp were sterilized with spirit then bags were kept in laminar air flow and switched on UV light for 30 minutes for sterilization.

Inoculation and Incubation

Bags were inoculated with equal amount of inocula (3 mm diameter bits) of fresh fruiting culture of (*Calocybeindica*) under aseptic condition on the same day considering the date of inoculation as zero day and incubated at room temperature (26 ± 4°C) for spawn growth. The inoculated bags were kept on B.O.D. incubator for observation and the days required for completion and texture of mycelium in bags were recorded. Further, these fresh spawn's bags were used for cultivation on paddy straw using polypropylene bags.

Preparation of Straw Substrate (Mushroom Bed)

Well dried paddy straw was chopped into 3-4 cm bits and then 10-12 kg straw was immersed in 100 litres water containing 100 ml formaldehyde and 10 gmbavistin to sterilize for 16-18 hours. Sterilization was done in 4 × 3 × 2.5 ft. size hauze. Then the mouth of the hauze was covered with polythene sheet to avoid release of the gases coming out from formaldehyde. Next day in the morning sterilized straw was taken out and drained off excess water by keeping on wire mesh frame and spread over on polythene sheet to dry for 1 to 1.5 hours depending on the prevailing weather condition. The moisture content of the straw was kept at 65-70 per cent. It was tested by palm method by squeezing the handful of straw. Then the straw was ready for spawning.

Spawning and Spawn Running

Polypropylene bags of 60 cm × 40 cm of 100 gauges were used. The bottom of the bag was tied with a rubber band to make a cylindrical shape to the bed. Then the bag is sterilized with spirit dipped cotton by swapping and then the bag was turned over so that the tied portion comes inside. Bottom of the bag was slightly widened. The bag was filled with alternate layers of straw (1.5 kg sterilized dry straw per bag) and spawn (300 g/kg of dry straw). Press it with palm to let the air go out. The bag was then tied with a rubber band along with a label of the species and date of spawning. About 10-15 holes were made into the polythene bags for the exchange of air and gases. Spawned bag was stacked in racks which were arranged in spawn running room. During spawn running period, temperature of 26 ± 4°C was maintained. These partially controlled conditions were maintained for 20 to 25 days for complete spawn running period when whitish cottony mycelia growth completely covered the straw in polythene bags. The polythene bags were cut into two halves with a hacksaw blade. After cutting of bags, casing soil were applied to a height of 2 cm above the newly exposed surface of the bags.

Preparation of Casing Mixture and Casing

Ten days after spawning, casing mixture were prepared in the ratio of 1:1 by using Garden soil (pH 5.3) and two years old farmyard manure (FYM, pH 6.45). Garden soil was taken from the glasshouse field, Department of Plant Pathology and their pH was analysed by using Elico-pH meter in the laboratory of the Department of Soil Science and Agricultural Chemistry. The casing media were prepared by through mixing of the selected substrates in the proper ratio and was chemically sterilized by spraying with 2 per cent formalin and then covered with polythene sheet for 3 days. The media were turned on alternate days for 4 days to remove the fumes of formalin from the casing mixture. The selected substrates taken to prepare casing media were mixed in volume-by-volume basis and slightly moistened after casing and then applied to create casing layer thickness of 2.0 cm. After casing beds were kept on racks in cropping room for fruiting. During this period the temperature and humidity were maintained. Observations were recorded.

Cropping Room

The temperature and relative humidity for fruiting were kept 30-35°C and 80-85 per cent, respectively. After casing of beds ventilation was reduced. Watering was done two times a day by a hand sprayer and it was withheld a day before harvesting. The yield data were recorded for a period of 35 days.

Harvesting

Pinheads appeared after casing and harvesting was done one week after pinning. Fully matured sporophore of milky white mushroom were harvested from the beds and fresh weight was determined immediately. Likewise, each bed was harvested for two croppings in period of 26 days after the first harvesting.

Observations Recorded

Yield attributing characters included were:

- Spawn run (days),
- Number of sporophores (No.)
- Size and Weight of sporophores

- Length of stipe (cm)
- Diameter of stipe (cm)
- Diameter of pileus (cm)
- Weight of sporophores
- Mushroom yield per bed

Number, size, weight of sporophores length, diameter of stipe and diameter of pileus and yield were recorded after harvest of mushroom.

Biological Efficiency (%)

Biological efficiency of mushroom was calculated by using a formula as recommended by Chang and Miles (1989).

$$\text{Per cent biological efficiency} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

RESULTS AND DISCUSSIONS

The yield of *C. indica* varied significantly when then spawn was prepared using different substrates.

Growth and Yield Parameters

Different grain substrates affected the growth and yield parameters such as spawn run (days), pinhead initiation (days) after casing, number of pinhead per bed, number of sporophore per bed, yield per bed (g) and biological efficiency. Shortest period (20.50 days) was observed in T_2 (jowar grain) which was found statistically at par (20.75 days) with T_1 (wheat grain). Longest period (22.75 days) was observed in T_4 (maize grain).Shortest period (6.50 days) was observed in T_2 (jowar grain) which was found statistically at par (7 days) with T_1 (wheat grain) and T_6 (bajra grain). Longest period (7.57 days) was observed in T_4 (maize grain). Maximum number of pinhead (22.75) per bed was observed in T_2 (jowar grain) which was found statistically at par (22.50) with T_1 (wheat grain) and T_6 (bajra grain). The maximum number of sporophores (15.75) per bed was harvested in T_3 (ragi grain) which was found to be significantly superior to other treatments. This was followed by 14.75 in T_1 (wheat grain) and T_2 (jowar grain). Minimum number of sporophores (12.50) was harvested in T_4 (maize grain). The maximum yield (1586.21 g) per bed was recorded in T_2 (jowar grain) which was found statistically at par (1521.75 g) with T_1 (wheat grain). Minimum yield (1031.37 g) per bed was recorded in T_4 (maize grain). Maximum biological efficiency (105.74%) was recorded in T_2 (Jowar grain) followed by 101.45 per cent in T_2 (wheat grain) **(Table 1 and Plate 1, Figure 1, 2).**

The days for pinhead initiation after casing were earlier in the case of spawn prepared using Jowar grain (6.50 days) followed by wheat (7.00 days) and Bajra grain (7.00 days). Similar results were reported by Doshi*et al* (1989) who observed that early fruit body production was noticed on sorghum (Jowar) grain spawn. The variation in the colonization in different substrate could be due to the variation in the amount of moisture observed during boiling, which is one of the critical factors responsible for mycelial growth (Mehta, 1985). Spawn prepared from maize grain recorded maximum average pinhead initiation (7.57 days), least no. of pinhead/bed (16.25) and average no. of sporophore (12.50), and

minimum yield (1031.37g), biological efficiency (68.75%) when compare to other spawn substrate. The yield and biological efficiency were maximum in the spawn prepared from Jowar grain which recorded 1586.21 g/bed and 105.74 percent, respectively. This Jowar grain spawn was statically superior to the spawn prepared from other grains. Maximum no. of sporophore/bed was recorded from the spawn prepared from Ragi grain (15.75). This was followed by Jowar and wheat grain which recorded 14.75 average no. of sporophore/bed each. Rangad and Jandaik (1977a) found maximum yield with sorghum spawn in different *Pleurotus sp*. Sivaprakasan and Kandaswamy (1981) obtained good yield of *P. sajor-caju* with sorghum and pearl millet.

Size and Weight of Sporophore

The size and weight of sporophore and fruiting bodies of *Calocybeindica* were affected with different grain based spawn. The result showed that the length of stipe, diameter of stipe and diameter of pileus in different treatments ranged from 10.69 cm to 14.96 cm, 6.27 cm to 8.20 cm and 9.45 cm to 11.90 cm, respectively.T2 (Jowar grain based spawn) recorded maximum length of stipe (14.96 cm), diameter of stipe (8.20 cm) and diameter of pileus (11.90 cm) which was found statistically at par with T_1 (wheat grain). Maximum weight of sporophore (107.54 g) was recorded in T_2 (jowar grain) which was found statistically at par (103.17 g) with T_1 (wheat grain). Minimum weight of sporophore (82.51 g) was recorded in T_4 (maize grain) **(Table 2, Plate 2, 3).**

The jowar grain based spawn gave highest net benefit-cost ratio of 3.12 followed by wheat grain based spawn with 2.89. Least net benefit-cost ratio (1.70) was obtained by maize grain based spawn **(Table 3).**

CONCLUSIONS

The production of mushroom largely depends on quality of spawn which is based on substrate. In the present investigation amongst the various spawn substrates evaluated, jowar grain was found to be the best spawn substrate for early spawn run, which took only 20.50 days for complete mycelia growth followed by wheat grain. The variation in the colonization of different substrates could be due to the variation in the amount of moisture observed during boiling, which is one of the critical factors responsible for mycelia growth (Mehta, 1985). The size and weight of sporophores, yield and biological efficiency were found maximum in jowar grain based spawn. Similar results were reported by Senthilnambi*et al.* (2011b), Doshi*et al.* (1989) and Lakshmipathy*et al.* (2011). Best growth of *Pleurotus* on jowar grain based spawn was also observed by Kumar *et al.* (1975), Kapoor (1989), Sivaprakasham and Ramaraj (1991) and Rathaiah and Swargiary (1994). Sivaprakasham and Kandaswamy (1981) and Chauhan and Pant (1988) observed superiority of jowar grain based spawn followed by wheat grain based spawn. Kumar *et al* (1975) reported that jowar grain gave better growth of mycelium after mixing 2% gypsum and 6% calcium carbonate with the boil grain by weight. Chauhan and Pant (1988) reported that Jowar and Bajra was found to best followed by wheat grain. Kapoor (1989) reported that wheat and sorghum grain were mostly used as spawn substrates for oyster mushroom. Rathaiah and Swargiary (1994) reported that out of sorghum, pearl millet, wheat and Maize grains as spawn base, sorghum was the best followed by pearl millet for Pleurotus spawn production. Santhilnambi*et al* (2011 b) reported that out of maize seed, cottonseed, sorghum seed, horsegram, blackgram, cowpea and ragi seed as spawn base, sorghum seed was the best followed by Ragi seed for milky mushroom spawn production. It was concluded that jowar grain was found as best spawn substrate and yield followed by wheat grain in respect of size (length of stipe, diameter of stipe and pileus) and weight of sporophores and yield and biological efficiency, though highest number of sporophores recorded by the use of ragi grain based spawn. The maize grain based spawn required longest

period for complete spawn run and recorded lowest yield when compared to other spawn substrates.

REFERENCES

1. *Crisan, E.V. and Sands, A. (1978). Nutritional value of edible mushrooms, In: The Biology and Cultivation of Edible Mushrooms. (Chang, S.T. and Hayes, W.A., Eds.) Academic Press, London pp 137.*
2. *Chadha, K.L. and Sharma, S.R. (1995). Mushroom Research in India: History, Infrastructure and Achievements. Advances in Horticulture,**13**: 1-12.*
3. *Chang, S.T. and Miles, P.G. (1989).Biology and Cultivation of Edible Mushrooms. Academic Press, London. pp. 265-274*
4. *Chauhan, S. and Pant, D.C. (1988). Effect of spawn substrates and storage conditions on sporophore production in Pleurotussajor -caju.Indian J. Mycol. Pl. Pathol.**18** (3): 231-234.*
5. *Doshi, A., Munot, J.F. and Chakravarti, B.P. (1988).Nutritional status of an edible mushroom Calocybeindica P & C. Indian J. Mycol. Pl. Pathol.**18**: 301-302.*
6. *Kumar, S., Seth, P.K. and Munjal, R.L. (1975).Studies on quantities of gypsum and calcium carbonate singly and in combination of spawn production of Agaricusbisporus. Indian J. Mush.**1** (2): 27.*
7. *Lakshmipathy, G., Tayakumar, A., Abhilash, M. and Raj, S.P. (2011).Optimization of growth parameters for increased yield of the edible mushroom Calocybeindica. African J.Biotech.**11** (11): 7701-7710.*
8. *Mehta, K.B. (1985). Studies on physiology and cultivation of Pleurotussapidus (Schulzer) Kalch. Ph. D. Thesis, HPKVV, Solan, India.*
9. *Purakayastha, R.P. and Chandra, A. (1985). Manual of Indian Edible mushrooms. Today and Tomorrows Printers and Publishers, New Delhi, pp. 192-194.*
10. *Purkayastha, R.P. and Nayak, D. (1979). Effect of substrates and spawn density on the production and protein content of fruiting of Calocybeindica. MushroomJ. Tropics.**30** (7): 132-134.*
11. *Purkayastha, R.P. and Nayak, D. (1981). Development of cultivation method and analysis of protein of promising edible mushroom Calocybeindica P & C. Mushroom Sci.**11**: 697-713.*
12. *Rangad, C.O. and Jandaik, C.L. (1977 a). Effect of different spawn substrates and storage condition on yield of Pleurotus species.Indian J. Mushroom, **3**:1-15.*
13. *Rathaiah, Y. and Swargiary, M. (1994).Use of parboiled paddy as spawn substrate for oyster mushroom (Abstr.).Mushroom Res.**3** (1): 45.*
14. *Senthilnambi, D., Eswaran, A. and Balabaskar, P. (2011b). Impact of different spawn substrates on yield of Calocybeindica. African J. Agri. Res. **6**(12): 3946-3948.*
15. *Sivaprakasham, K. and Kandaswamy, T.K. (1981).Effects of different types of spawn on sporophore production of Pleurotussajor-caju. Madras Agric. J.**68**: 178-182.*

APPENDICES

Table 1: Screening of Different Spawn Substrates for Spawn Production of *Calocybeindica*

Treatments	Average Spawn Run Days	Average Pinhead Initiation (Days) after Casing	Average No. of Pinhead/ Bed	Average No. of Sporophore/Bed	Average Yield/Bed (gm)	Biological Efficiency (%)
T_1 - Wheat grain	20.75	7.00	22.50	14.75	1521.75	101.45
T_2 - Jowar grain	20.50	6.50	22.75	14.75	1586.21	105.74
T_3 - Ragi grain	21.50	7.50	19.75	15.75	1362.84	90.85
T_4 - Maize grain	22.75	7.57	16.25	12.50	1031.37	68.75
T_5 - Paddy grain	21.75	7.50	18.50	13.25	1160.46	77.36
T_6 - Bajra grain	21.00	7.00	22.50	14.50	1440.18	96.01
SEm ±	0.24	0.19	0.27	0.27	30.49	
CD at 5%	0.72	0.58	0.81	0.82	91.30	
CV%	3.92	5.75	2.62	3.91	7.16	

Table 2: Effect of Different Spawn Substrates on Size and Weight of Sporophore of *Calocybeindica*

Treatments	Average Length of Stipe (cm)	Average Diameter of Stipe (cm)	Average Diameter of Pileus (cm)	Average Weight of Sporophore (gm)
T_1 - Wheat grain	14.55	7.84	11.55	103.17
T_2 - Jowar grain	14.96	8.20	11.90	107.54
T_3- Ragi grain	13.58	6.85	10.62	86.53
T_4 - Maize grain	10.69	6.27	9.45	82.51
T_5 - Paddy grain	11.75	6.59	9.83	85.96
T_6 - Bajra grain	13.60	7.35	10.80	102.87
SEm ±	0.23	0.21	0.29	1.52
CD at 5%	0.69	0.63	0.89	4.57
CV%	4.75	5.57	5.24	3.05

Table 3: The Economics of the Production of Fresh Mushroom per Bed of *Calocybeindica* Using Different Spawn Substrates

Treatments	Total inputs (Rs)	Yield/bed (g)	Gross income (Rs.)	Net benefit (Rs.)	Net benefit-cost ratio (Rs.)
T_1 - Wheat grain	39.05	1521.75	152.17	113.12	2.89
T_2 - Jowar grain	38.45	1586.21	158.62	120.17	3.12
T_3 - Ragi grain	36.05	1362.84	136.28	100.23	2.78
T_4 - Maize grain	38.15	1031.37	103.13	64.98	1.70
T_5 - Paddy grain	37.25	1160.46	116.04	78.79	2.11
T_6 - Bajra grain	39.95	1440.18	144.01	104.06	2.60

paddy straw (chopped) @ Rs. 6 /kg; Jowar grain @ Rs. 15/kg; Wheat grain @ Rs. 17/kg; Ragi grain @ Rs. 7/kg; Maize grain @ Rs. 14/kg; Paddy grain @ 11/kg, Bajra grain @ Rs. 20/kg;

Spawn @ Rs. 10.50/bag (300 g wheat grains based spawn).

Polythene bag @ Rs. 2/bag.

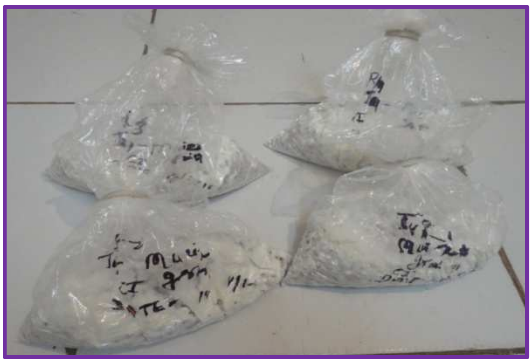
Miscellaneous charges @ Rs. 3/bag

Mushroom selling price @ Rs. 100/kg fresh mushroom.

T_1 - Wheat Grain

T_2 - Jowar Grain

T_3 - Ragi Grain



T_4 - Maize Grain

T_5 - Paddy grain

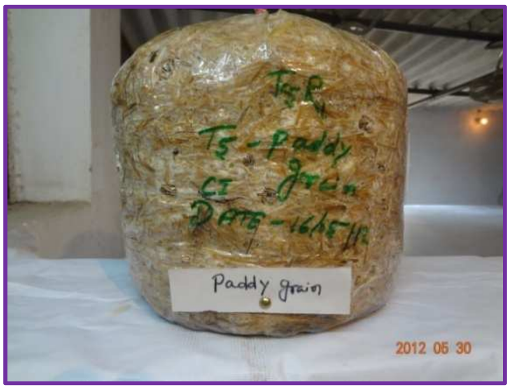
T_6 - Bajra Grain

Plate 1: Different Spawn Substrates for Spawn Production of *Calocybeindica*

T_1 - Wheat Grain

T_2 – Jowar Grain

T_3 – Ragi Grain **T_4 – Maize Grain**



T_5 – Paddy Grain **T_6 – Bajra Grain**

Plate 2: Fully Grown Mycelium of *Calocybeindica* on Paddy Straw Substrates Spawned With Different Spawn Substrates

T_1 – Wheat Grain

T_2 – Jowar Grain

Plate 3: Fruiting Bodies of *Calocybeindica* on Paddy Straw Substrates Spawned With Different Spawn Grains

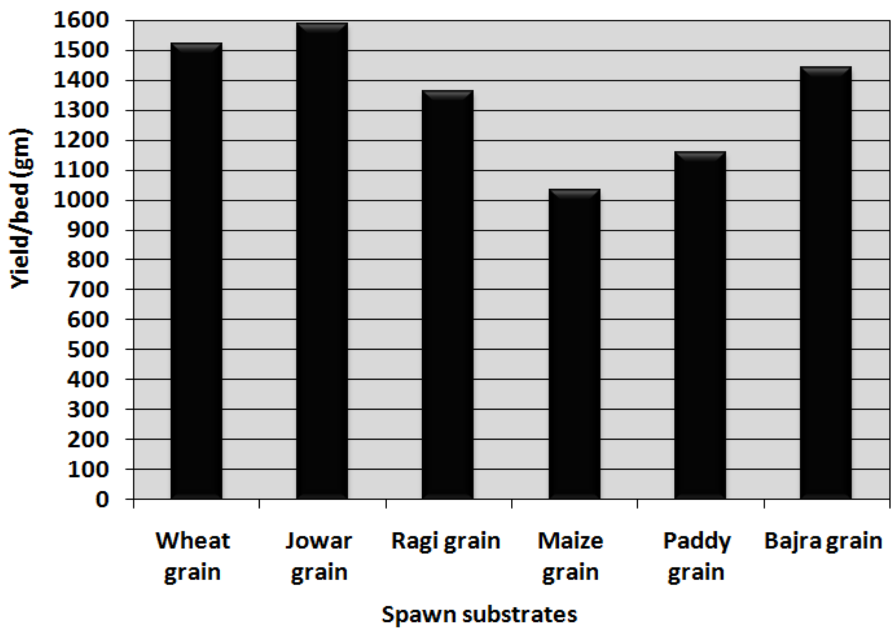


Figure 1: Effect of Different Spawn Substrates on the Yield of *Calocybeindica*

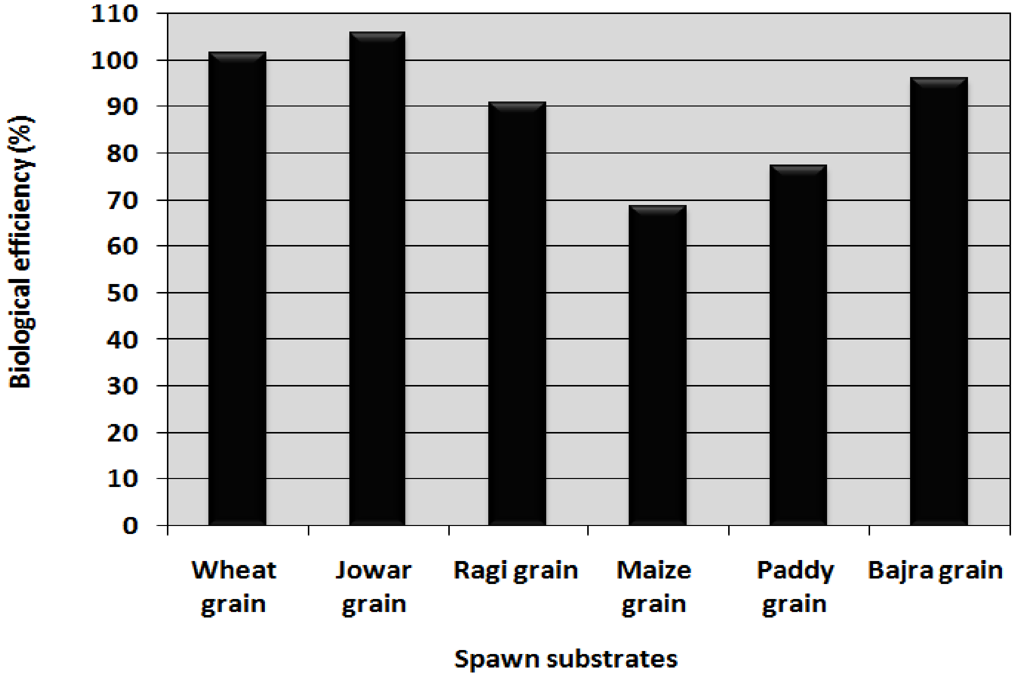


Figure 2: Effect of Different Spawn Substrates on the Biological Efficiency of *Calocybeindica*